PHARMACOLOGY OF THE AUTONOMIC NERVOUS SYSTEM[1,2]

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This review is concerned chiefly with the pharmacological aspects of synaptic transmission at various junctional sites in the peripheral autonomic nervous system. Included are a survey of recent findings concerning the interactions of drugs with receptor sites on electrogenic membranes, the effects of chemical agents on the synthesis, storage and release of transmitter agents, and the effects of drugs on the mechanisms involved in the termination of transmitter action.

Transmission in Autonomic Ganglia

According to the prevailing concept of transmission in autonomic ganglia, the invasion of the preganglionic nerve terminals by the nerve impulse effects the liberation of ACh which, in turn, diffuses across the synaptic cleft and impinges upon postsynaptic elements to initiate electrical activity. Although there is no reason for challenging this concept of transmission, recent studies from several laboratories have emerged which underscore the complexities of the process.

Presynaptic site of action of drugs.—In a general proposal concerning the action of ACh on various sensory and motor junctions, Koelle (1, 2) has suggested that ACh liberated endogenously from the nerve endings of the superior cervical ganglion during transmission acts upon the nerve terminals to liberate additional transmitter. As a corollary to the proposal, the role of presynaptic acetylcholinesterase (AChE) is to limit the presynaptic action of the transmitter. These proposals were based in part on the findings that AChE in sympathetic ganglia is localized primarily in the presynaptic nerve filaments (3), and that chronically denervated ganglia were less sensitive to injected ACh or carbachol than innervated ganglia (4). Consistent with this proposal is the finding by McKinstry *et al.* (5) that carbachol evoked the release of ACh from resting perfused ganglia.

[1] The survey of the literature pertaining to this review was concluded May, 1962.

[2] Abbreviations used in this chapter include: ACh (acetylcholine); AChE (acetylcholinesterase); TEA (tetraethylammonium); C_6 (hexamethonium); *d*TC (*d*-tubocurarine); DFP (dipropyl phosphorofluoridate).

[3] The work of the author, described in this report, was supported in part by U.S.P.H.S. Grant B-3434.

[4] The author wishes to express his appreciation to Dr. George B. Koelle for many helpful suggestions in the preparation of this manuscript, and to Drs. A. Komalahiranya and C. Takeshige for their contribution in the author's laboratory.

Also consistent with the above proposal is the enhancement of the postganglionic responses to injected ACh and carbachol which occurred following conditioning of the ganglia with repetitive preganglionic volleys (6). Although the nature of the changes produced by the conditioning procedure is obscure, there is general agreement that the alterations are confined primarily to the nerve endings. Curtis & Eccles (7) have presented an excellent discussion of the changes produced at junctional sites by prejunctional repetitive stimulation.

A major difficulty in accepting this concept of a presynaptic site of action of ACh is the failure of ACh and other cholinomimetic drugs to consistently activate retrograde firing along the preganglionic nerve either before or after the administration of anti-esterase agents (4, 8) or after repetitive preganglionic stimulation (6). This occurs along the motor nerve to skeletal muscle under similar conditions, presumably as the result of drug action at a presynaptic site (9 to 11). Inconstant retrograde preganglionic firing evoked by ACh has been described in cats (4, 12) and rabbits (8) and has been attributed reasonably to the activation of ganglion cells distributed along the preganglionic nerve (8). Nonetheless, the failure of cholinomimetic agents to activate true antidromic discharges does not compromise necessarily the concept of a presynaptic site of action of the ganglionic stimulants. It is conceivable that these agents can act on the nerve endings to release the transmitter substances without producing an electrogenic effect sufficient to evoke retrograde firing. For example, facilitation by tetraethylammonium (TEA) of the liberation of ACh from the nerve endings of sympathetic ganglia (13) and neuromuscular junction (14) occurred without producing concomitant retrograde firing along the motor nerve.

Also, during the past two years, the actions of a number of other chemical agents on the presynaptic nerve endings of autonomic ganglia have been described. Part of the blocking action of nicotine on transmission through the superior cervical ganglion has been attributed to a depressant action of nicotine on the liberation of ACh from the nerve terminals (15, 16). Unlike that produced by hexamethonium (C_6) the blockade by nicotine of ganglionic response in preganglionic volleys was of significantly longer duration than the blockade of the response to injected ACh (16). On the other hand, inhibition of the release of ACh from perfused ganglia by various central nervous system depressants was considered not to be an important contributing factor in the blockade of ganglionic transmission produced by these agents (17). These latter findings are consistent with the earlier study by Exley (18). In addition to the aforementioned enhancement of the liberation of ACh by TEA (13, 14); epinephrine (19), barium ions (20), and a series of nitroparaffin compounds (21) facilitated or caused the liberation of ACh from autonomic ganglia and the nerve terminals of postganglionic parasympathetic nerves. Whereas epinephrine depressed the liberation of ACh in ganglia perfused with Locke solution, an increase in the amount of

ACh released into the effluent occurred when epinephrine was administered to ganglia perfused with plasma. An unidentified factor (not epinephrine) in plasma has been reported to be essential for the efficient release of ACh (19). Barium ions appear to act as suitable substitutes for calcium ions in perfused ganglia (20). The nitroparaffin compounds, nitromethane in particular, caused contraction of the guinea pig ileum which was depressed partially by C_6. Contraction produced by nitromethane diminished with repeated applications but, significantly, was restored by exposure of the gut to ACh. It was suggested that exogenous ACh replenished the stores of endogenous ACh which were presumably depleted by the nitroparaffin compounds (21).

Pharmacological analysis of cholinoceptive sites on ganglion cell membranes. —The stimulation of ganglia by agents such as histamine and 5-hydroxytryptamine is known to be resistant to blockade by the traditional ganglionic blocking agents. Accordingly, such activity is attributed usually to the interactions of the above agents with noncholinoceptive sites in the ganglia. However, stimulation of sympathetic ganglia by the pressor agent, 4-(m-chlorophenylcarbamoyloxy) - 2 - butynyltrimethyl ammonium chloride (McN-A-343), although resistant to blockade by C_6, was sensitive to blockade by atropine (22). Further studies indicated also that the action of McN-A-343 was not blocked by nicotine (23), nor 3,6-bis(3-diethylaminopropoxy) pyridazine *bis*-methiodide (Win 4981) (24), a compound with actions like those of hemicholinium (HC-3). Thus, the blockade by atropine of the action of McN-A-343 on the ganglia indicates the existence of distinctive types of cholinoceptive sites on the ganglion cells.

Further evidence for pharmacologically distinctive types of ganglionic receptors for ACh liberated endogenously has been presented by Eccles & Libet (25). Previously Eccles (26) had demonstrated that the response of isolated curarized superior cervical ganglia of rabbits to a preganglionic volley was characterized by a complex waveform with three components: (*a*) initial negative potential (N), (*b*) the positive potential (P), (*c*) and a late negative potential (LN). Higher concentrations of curare depressed the amplitude of the N potential and increased that of the LN potential. Further analysis of the effects of drugs on these potentials revealed that they were blocked by botulinus toxin and that the LN potential was sensitive to blockade by atropine. (The effects of drugs on the P potential will be discussed below.) Consequently, the following model of transmission was proposed. All of the potentials were due to ACh liberated from the nerve endings which combined with curare- and atropine-sensitive sites on the ganglion cells to give rise to the N and LN potentials, respectively.

A similar pattern of blockade by *d*-tubocurarine (*d*TC) and atropine on transmission has been observed in diisopropyl phosphorofluoridate (DFP)-treated ganglia of cats (27). Asynchronous postganglionic firing occurred in resting ganglia, but not in resting denervated ganglia, treated with DFP (4, 27). *d*-Tubocurarine, administered in amounts which blocked completely the responses of the DFP-treated ganglia to nerve stimulation or injected

ACh, caused a further activation of the DFP-discharge. Conversely, the DFP discharge was sensitive to blockade by atropine injected in doses which had no apparent effect on the postganglionic spikes produced by preganglionic stimulation or injected ACh. It is noteworthy also that the blockade of the discharge by atropine persisted for hours, whereas the blockade of the response to nerve stimulation which occurred after higher doses of atroprine and atropine-like drugs was transient (27, 28). Similarly, the DFP-discharge was more sensitive to blockade by procaine than the other ganglionic responses studied. Emmelin & MacIntosh (29) have described a "late" firing of ganglion cells following repetitive stimulation of ganglia perfused with media containing anti-esterase agents which was not blocked by C_6 or *d*TC. Furthermore, they reported that no ACh appeared in the effluents of the ganglia during the period of "late" firing. In a later study, Birks & MacIntosh (19) were able to detect small amounts of ACh in the perfusate of resting ganglia treated with large doses of anti-esterase agents. In view of these findings, it was suggested that the mechanism underlying the DFP-discharge differed from the usual forms of transmission in sympathetic ganglia (27).

Treatment of the ganglia with large doses of physostigmine or repetitive stimulation of ganglia treated with smaller doses of physostigmine also evoked asynchronous activity which was sensitive to blockade by atropine but not by *d*TC or mecamylamine (30). The responses to ACh of ganglia treated with physostigmine or ganglia conditioned with tetanic volleys were characterized by an "early" and "late" phase. The "early" phase of the response was blocked by *d*TC; the "late" phase by atropine. Whatever relationship these phases may have to the N and LN ganglionic potentials described by Eccles & Libet (25) as having corresponding sensitivities to *d*TC and atropine, respectively, is not known.

The effects of inorganic ions and veratrine on the DFP-discharge also have been studied (31). The enhancement by veratrine and the blockade by cadmium chloride of the discharge were attributed to a presynaptic action of the drugs. A similar prejunctional site of action at the neuromuscular junction has been described for these agents (32, 33).

The pressor response evoked by neostigmine or physostigmine in dogs pre-treated with C_6 or other ganglionic blocking agents has been explained on the basis of a ganglionic stimulating action of the anticholinesterase agents (34, 35). Since this response was blocked by small doses of atropine, the mechanism involved is conceivably the same as that considered above for DFP-treated ganglia. The antagonism by neostigmine, but not by physostigmine, of blockade of the superior cervical ganglion by C_6 also has been described (36). In addition, neostigmine injected into the arterial supply of of both normal and denervated ganglia was found to produce contraction of the nictitating membrane. Furthermore, neostigmine evoked a postganglionic discharge in denervated as well as in normal ganglia, which was in turn depressed by small doses of atropine (37). The importance of the presynaptic

nerve terminals in this response was indicated by the finding that a 20-fold greater dose of neostigmine was required to evoke the firing in denervated ganglia than in normal ganglia. Thus, it appears that neostigmine may have at least two actions on sympathetic ganglia: to increase or preserve the output of transmitter, and to unmask a second, pharmacologically distinctive cholinoceptive site. Either action of neostigmine could account for the antagonism of the ganglionic blockade by C_6.

On the other hand, studies by Lesic & Varagic (38) of the hypertension produced by physostigmine in rats have indicated a central action of this drug, in confirmation of the authors' earlier studies.

Pharmacological evidence for an adrenergic inhibitory system in sympathetic ganglia.—Gertner (39) has reported that various inhibitors of monoamine oxidase blocked completely the response of perfused ganglia to preganglionic stimulation but had no effect on the response to injected ACh. Furthermore, these agents had no effect on the release of ACh from the nerve endings. Thus, the curious situation was found in which transmission was blocked, but transmitter output and the sensitivity of the ganglion cells to the transmitter were normal. The search for inhibitory substances in the effluent of the ganglia was unsuccessful. No satisfactory explanation of these findings is apparent.

Potentiation by reserpine or an adrenergic blocking agent of the response of the superior cervical ganglia to preganglionic volleys has been attributed to the depletion or blockade, respectively, of norepinephrine in the ganglia (40). [These findings with reserpine are not in agreement with earlier reports (41, 42).] Similarly, the depression by dibenamine of the P potential of curarized ganglia has been explained on the basis of antagonism between dibenamine and endogenous catecholamines (25). The P potential was depressed also by atropine. Accordingly, Eccles & Libet suggested that ACh liberated from the nerve endings acted upon chromaffin stores (the atropine-sensitive site) to release catecholamines which, in turn, act upon the ganglion cells to initiate the P potential (the dibenamine-sensitive site). Consequently, the suggestion has been made that catecholamines modulate transmission in sympathetic ganglia (25, 40).

Gamma-aminobutryric acid (GABA) has been shown to possess weak ganglionic blocking activity. Curiously, it depressed transmission in the inferior mesenteric ganglion of the cat, but not in the superior cervical ganglion (43). Gamma-aminobutyric acid also depressed transmission in superior cervical ganglia of rats infected with pseudorabies virus (44).

Heterogeneity of cell groups within sympathetic ganglia.—At least four distinct cell groups in the superior cervical ganglia have been identified by electrophysiological analysis of the compound action potential of the cervical sympathetic trunk, the ganglionic potentials, and the action potentials of postganglionic nerves (45). However, only a limited number of studies have been performed on the actions of drugs on the several cell groups. Shaw and

associates (46, 47) observed that the blockade by ganglionic blocking agents of the responses of the nictitating membrane and vasomotor fibers of the ear to repetitive stimulation of the preganglionic nerve differed in intensity and duration. These findings were considered indicative of functional heterogeneity within the ganglia (47). Similar differential blockade by various agents of cell groups of the rat stellate ganglion *in vitro* (48) and cat superior cervical ganglion *in situ* (49) has been described. In addition, treatment of the superior cervical ganglia of cats with botulinus toxin prevented the pupillary response to preganglionic stimulation but had no effect on the response of the nictitating membrane (50) or external carotid nerve (4). However, it is important to remember that since many of these effects differed only quantitatively, they may reflect uneven distribution of the drugs rather than inherent functional differences among the cell groups.

Another example of heterogeneity in sympathetic ganglia is the difference in the AChE content of the ganglion cells. Sjoqvist (51, 52) has reported further on the results of very precise counting of histochemically localized AChE containing cells of sympathetic ganglia. Correlation was found between the number of heavily stained ganglion cells of some ganglia (L_6, L_7, and S_1) and the outflow of cholinergic fibers to sweat glands.

Biochemical aspects of transmission in sympathetic ganglia.—The metabolism of ACh in the superior cervical ganglia of cats has been studied in detail by Birks & MacIntosh (19, 53). It was concluded that about 85 per cent of the ACh which can be extracted from the ganglia was derived from the nerve endings and existed in two fractions, one of which was more easily liberated by the nerve impulse than the other. Normally, the rate of release of ACh does not exceed its rate of synthesis. However, if choline was absent from the extracellular fluid or if hemicholinium (HC-3) was added, depletion of the stores of ACh occurred. As mentioned above, an unidentified factor in plasma was required for the efficient synthesis or liberation of the transmitter.

The results of a number of studies have indicated that AChE and choline acetylase of cholinergic neurons are synthesized in the cell body and transported to the peripheral arborizations of the neuron by means of axoplasmic currents. Recent studies by Koenig & Koelle (54) of the regeneration of AChE in the perikarya and nerve trunks of various cholinergic neurons following the administration of irreversible antiesterase agents indicated that the regeneration of AChE in the periphery proceeded at rates independent of that in the cell bodies and occurred uniformly along the nerve trunk. Evidence in favor of the centrifugal migration of AChE by axoplasmic currents has been reported also (55). In the latter study, the concentration of AChE activity in segments of peripheral nerve was reported to decrease linearly with the distance of the segment from the cell body of origin. Most likely, differences in technique are responsible for the conflicting findings of the two studies.

There is little doubt that cholinergic neurons play important roles in the

metabolism of AChE in effector organs. Surgical innervation with motor nerve of regions of skeletal muscle at which AChE is normally absent induced the formation of concentrated packets of enzyme on the postjunctional side of the artificial junction (56). The source of the newly formed enzyme (nerve, muscle, or satellite cell) is not known. On the other hand, in embryonic chick heart functional levels of cholinesterase as well as adrenoceptive and cholinoceptive sites were observed prior to the development of vagal innervation (57).

Attempts to isolate chemically the receptor substance of neural structures for ACh have been focused primarily on fractionation procedures and measurements of the capacity of various fractions for binding curare and related substances (58). A protein recovered from ammonium sulfate fractions of electric tissues of *Electrophorous electricus* which precipitated from solution when treated with curare was reported initially to be the receptor for ACh (58, 59). Somewhat later, however, this interpretation was changed and the protein was assigned a less specific role in transmission or conduction processes (60).

Several interesting studies concerning changes in the phospholipid and oxidative metabolism of sympathetic ganglia during transmission and treatment with ACh have been reported during the last few years. Incubation of slices of stellate ganglia of cats with ACh caused a marked increase in the incorporation of isotopic phosphorous in phosphoinositide and phosphatidic acid fractions of the ganglia (61). In conflict with this, prolonged repetitive stimulation of the preganglionic nerve of the superior cervical ganglion resulted in an increase only in the phosphatidyl inositol fraction (62). No effect on phosphatidyl choline, phosphatidyl ethanolamine, or phosphatidic acid was observed in the latter study. In addition, stimulation of autonomic nerves or the application of sympathomimetic or cholinomimetic drugs to the pancreas and salivary glands increased the turnover of phospholipid phosphorous and phosphatidopeptide (63, 64). The oxidation of glucose by resting and active ganglia has been restudied(65). It was concluded that the oxidation of glucose accounts satisfactorily for most of the increase in oxygen utilization that was observed during activity. The results of this study were acknowledged by the authors to be somewhat at variance with an earlier report (66). In view of the technical complexities involved in attempts to correlate biochemical changes with activity, conflicting findings are not really surprising.

Treatment of sympathetic ganglia with agents which complex sulfhydryl-groups produces marked alterations in the ganglionic responses to drugs. Cadmium chloride and *p*-chloromercuribenzoate blocked the response to preganglionic nerve stimulation and enhanced the response to injected ACh in perfused ganglia (67). It is unfortunate that the effects of these agents on the output of ACh were not studied. The sulfhydryl inhibitor N-ethylmaleimide (NEM), in addition to producing the changes described above for cadmium, evoked also a persistent asynchronous pre- and postganglionic

discharge and unmasked an excitatory component in the actions of *d*TC on the ganglia. Unlike the anti-esterase agents, these actions of NEM were not antagonized by atropine (68). In addition, an interaction with sulfhydryl groups has been suggested as the basis for the blockade by C_6 and nicotine of transmission in sympathetic ganglia (69). The relationship between these findings and the role of sulfhydryl groups, if any, in transmission in sympathetic ganglia is not clear.

Transmission in the ciliary ganglion.—Only a limited number of studies have been made on transmission in the ciliary ganglion (70 to 72). It was reported recently that transmission at this site was facilitated by low doses of standard ganglionic blocking agents (72). In addition, the ciliary ganglion was less sensitive than the superior cervical ganglion to blockade by some ganglionic blocking agents. Once again, the question of distribution of the drugs becomes an important aspect of determining whether this latter difference in the response to drugs indicates differences in the mechanism of transmission in the two ganglia.

Two forms of transmission, one mediated by electrical-coupling and the other by ACh, have been reported to occur in the ciliary ganglion of the chick (73). The electrically coupled transmission was characterized by poor rectification (a presynaptic potential was evoked by stimulation of postsynaptic elements) and resistance to blockade by *d*TC. To date, this type of transmission has not been described in mammalian ganglia.

Artificial vagal-adrenergic synapse.—The effect of drugs on the heterogeneous synapse formed by the reinnervation of denervated superior cervical ganglia of cats by the central ends of afferent vagal fibers as performed originally by deCastro (74), has been studied by Matsumura & Koelle (75). In successful preparations, stimulation of the vagus or inflation of the stomach produced mydriasis and contraction of the nictitating membrane. Thus, this synapse provides an excellent opportunity to study the process of transmission by sensory neurons. DeCastro (74) had reported earlier that since transmission at this synapse was not affected by locally applied physostigmine it was not mediated by ACh. Using the intra-arterial route of administration of drugs, Matsumura & Koelle (75) observed that physostigmine enhanced, and TEA blocked transmission in the vagally innervated sympathetic ganglion. From these and other pharmacological and histochemical observations, it appears that ACh is involved in the transmission process. However, the exact role of ACh is not readily apparent.

Transmission in the Adrenal Medulla

The most direct index of transmission in the adrenal medulla is the measurement of the output of catecholamines by the medullary cells in response to chemical agents or stimulation of the splanchnic nerve. Using this approach, Douglas *et al.* (76, 77) have studied the influence of calcium ions on the release of catecholamines produced by ACh, and compared the process to "excitation-contraction coupling" of muscle. According to this proposal,

ACh or potassium ions altered the medullary cells so that calcium ions entered in increased amounts, and initiated the release of the medullary hormones. The conditions for the release of catecholamines during nerve stimulation also have been described (78, 79). In the dog, the maximum output of catecholamines occurred when the splanchnic nerve was stimulated at a rate of 15 to 20 cps. In addition, the ratio of norepinephrine to epinephrine in the effluent was influenced by the rate of nerve stimulation.

Traditional ganglionic blocking and stimulating drugs have comparable actions on transmission in the adrenal medulla (79). However, the reversal by N,N-di*iso*propyl-N′-*iso*amyl-N′-diethylaminoethyl urea of the pressor response to ACh in atropinized dogs has been attributed to a specific blockade of the adrenal medulla (80). This compound has very weak ganglionic blocking activity. The pressor response in cats evoked by histamine was due primarily to stimulation of the adrenal medulla, whereas, that produced by pilocarpine was due to activation of ganglion cells (81).

Peripheral Actions of Ganglionic Blocking Agents

A considerable amount of controversy exists concerning the explanation of the potentiation by ganglionic blocking drugs of the pressor response evoked by catecholamines and other drugs. At one time, this phenomenon was generally explained primarily on the basis of the blockade of compensatory reflex mechanisms. However, at present it appears that more peripheral actions of some of the blocking agents also play an important role in the process (82, 83). The experimental observations cited below indicate clearly the complexities of this problem.

Contrary to the earlier findings in laboratory animals (82), C_6 administered intra-arterially had no effect on the vasoconstriction produced by the infusion of norepinephrine into the forearm of human subjects (84). Similarly, C_6 had no effect on the pressor responses of vagotomized spinal animals or on the contractile responses of isolated arterial strips to epinephrine (85). On the other hand, mecamylamine potentiated consistently the responses of isolated atria, arterial strips, and spinal animals to catecholamines and other excitatory agents (85, 86). To complicate matters further, potentiation of vasopressor drugs by TEA occurred with the arterial strips and spinal animals but not with the isolated atria. Thus, for some of the ganglionic blocking agents and for some of the peripheral neuro-effector junctions, a direct interaction of the blocking agent at the peripheral site occurs. Lum & Woodward (87) attributed the potentiation of pressor and depressor agents by ephedrine to both a blockade of reflex mechanisms and to a direct action at perhiperal sites. It is likely that more than one action is involved in the potentiation of pressor agents by ganglionic blocking drugs.

Transmission at Sympathetic Nerve Endings

Essentially all major aspects of the pharmacology, physiology, and biochemistry of the sympathetic nervous system have been discussed in great

detail in the official publication of the *Symposium* on *Adrenergic Mechanisms* which was held in March 1960 and sponsored by the Ciba Foundation (88) and in the *Symposium* on *Catecholamines* held in 1959 by the National Institutes of Health (89). Similarly, excellent accounts of the physiology of transmission in this division of the peripheral nervous system can be found in the series of handbooks of physiology published by the American Physiological Society (90, 91). Thus, the major task of the present review is to comment on important developments in this field which have occurred during the past two or three years. However, since the present comments represent the first review of some of the aspects of this subject to appear in *Annual Review of Pharmacology*, observations described in the earlier literature will be cited whenever necessary.

Electrophysiological analysis of drug action at sympathetic neuroeffector junctions.—Bülbring has advanced the hypothesis that the response of smooth muscle cells to epinephrine is dependent upon whether the predominent action of epinephrine is on the cell membrane or on cellular metabolic pathways which affect the membranes indirectly (92). According to this proposal, the direct action of epinephrine on the membrane results in contraction of the smooth muscle; the indirect action, in relaxation. In addition, both actions can be demonstrated in the same tissue (93, 94). For example, epinephrine reduced spontaneous electrical activity, abolished the conducted response to nerve stimulation, hyperpolarized the cell membrane, and increased phosphorylase activity of isolated taenia coli of guinea pigs bathed in a saline medium containing glucose. However, if glucose was removed from the medium or a metabolic inhibitor was added, then the actions of epinephrine were reversed.

One of the now classical signs of transmitter activity at junctional tissues is the occurrence of spontaneous miniature potentials (95). In general, drugs or procedures which modify the frequency of these potentials are considered to act on the nerve terminals; on the other hand, changes in amplitude indicate an interaction of the drugs at the postsynaptic elements. Recent studies by Burnstock & Holman have shown that stimulation of the hypogastric nerve to the vas deferens of guinea pigs resulted in a junctional potential which was composed of a number of miniature junctional potentials (96 to 98). The spontaneous potentials were not affected by atropine but were reduced in amplitude by yohimbine and piperoxan (97). Both the frequency and amplitude of the miniature potentials were decreased in preparations pretreated with reserpine (98). By analogy with studies of this type at the neuromuscular junction, it can be reasonably concluded that the miniature potentials were due to the liberation of quanta of norepinephrine from the adrenergic nerve endings.

Changes in membrane potential associated with the response of smooth muscle cells to stimulating drugs have been examined with similar techniques (99). The occurrence of diminished or enhanced responses to the drugs was

related to the polarization of the membrane at the time of application of the drug. In general, depolarization of the membrane was associated with potentiation of the response and hyperpolarization of the membrane with depression.

The effects of drugs on the electrical response of the nictitating membrane to a nerve volley indicate a dual innervation at this junction (100). The response of the membrane was characterized by a compound waveform consisting of two large potentials and a number of smaller oscillatory potentials. The first potential was increased by physostigmine, and blocked by scopolamine; the second potential was blocked by phenoxybenzamine, piperoxan, and reserpine. These findings were considered to indicate a dual, but distinct, cholinergic and adrenergic innervation to the nictitating membrane.

A cholinergic mechanism in adrenergic transmission.—The hypothesis by Burn & Rand that ACh is liberated at the terminals of sympathetic nerves and effects locally the liberation from the nerve endings or chromaffin stores of catecholamines which, in turn, activate the effector organs has been well documented and requires little amplification (1, 101 to 105). In essence, the concept is based on the sympathomimetic effect of ACh or nicotine on various organs in the presence of atropine and, conversely, on the cholinomimetic responses evoked in some organs by sympathetic nerve stimulation following drug-induced depletion of catecholamines from the nerve endings. Testing of the hypothesis is complicated by the presence of peripherally displaced ganglion cells which are susceptible to stimulation by ACh or nicotine, and the problem of dual, but distinct, sympathetic and parasympathetic supplies to some organs. In addition, there is the ever present problem of translating pharmacological evidence into terms of physiological significance. It is in this connection that the studies of Bülbring, in which it was shown that the functional state of the cell influences markedly the response to catecholamines, must be kept in mind. The fact that drugs such as reserpine may produce effects other than a depletion of transmitter (106 to 108) complicates further the analysis of experiments designed to test this hypothesis. These comments, however, are not meant to detract from the attractiveness or usefulness of the hypothesis.

The best evidence for an intermediary role of ACh in adrenergic transmission has been obtained from the studies of the actions of ACh on the spleen (109 to 112). In this organ, ACh has two actions, (*a*) a decrease in vascular resistance elicited by a small dose of ACh, which is sensitive to blockade by atropine, and (*b*) contraction of the spleen produced by larger doses of ACh, which is abolished by adrenergic blocking drugs, reserpine, C_6, or degeneration of the sympathetic nerve supply. Since it is highly unlikely that the latter response to ACh was due to stimulation of ganglion cells (110), it has been attributed to activation of the sympathetic nerve or chromaffin stores. In addition, ACh caused the liberation of norepinephrine

from the spleen (112). Finally, stimulation of the splenic nerve of cats pretreated with reserpine resulted in the appreance of ACh in the venous effluent of the spleen (111).

Additional support for this hypothesis has been obtained in studies of papillary muscle by Lee *et al.* (113), of embryonic chick heart by Lee & Shideman (115), and of the ileo-sphincter of rabbits by Jarrett (114). The excitatory actions of ACh, tetramethylammonium ions, and nicotine on these organs cannot be explained in terms of ganglionic stimulation. Consequently, Lee & Shideman, and Jarrett postulated that some unidentified structure which contains catecholamines, or a cholinergic junction, exists between the nerve endings and the tissue receptors. A similar site of action has been proposed to account for the sympathomimetic actions of 4-methyl-2 aminopyridine on the heart (116).

In addition to the use of reserpine and of other drugs which modify the liberation of transmitter from adrenergic nerve endings, hemicholinium (HC-3), which is known to interfere with the synthesis of ACh by intact nerves (117) has been used to examine this hypothesis. The hypothesis predicts that agents of this type should abolish transmission at adrenergic junctions. Hemicholinium was found to block the response to sympathetic nerve stimulation of the spleen (111), vas deferens (118), isolated uterus (119), colon (119, 120), perfused rabbit ear (119), bladder (120), and isolated atria of the cat (119). On the other hand, HC-3 had no effect, either *in vitro* (121) or *in vivo* (122), on the response of the nictitating membrane to nerve stimulation. Furthermore, evidence has been presented for a direct action of ACh on the nictitating membrane (123), and for a dual, but separate, innervation of the membrane (100). Thus, it seems unlikely that ACh plays a physiological role in adrenergic transmission at this site.

A viewpoint opposite to that expressed by Burn & Rand has been suggested by Gillespie & MacKenna (124) to explain the contraction which occurred during stimulation of sympathetic nerves to rabbit gut treated with reserpine. According to the hypothesis of Burn & Rand (125, 126), this cholinomimetic response to sympathetic nerve stimulation would be due to ACh liberated from sympathetic fibers. However, since the response was dependent upon the integrity of parasympathetic innervation and was not unmasked by traditional adrenergic blocking agents or 2,6-xylyl choline ether (TM-10), Gillespie & MacKenna concluded, by exclusion, that the contraction was due to the activation of the parasympathetic nerves by the sympathetic nerves.

Thus, in terms of transmission at autonomic neuro-effector junctions concepts have run the gamut from traditional dual cholinergic and adrenergic mechanisms to cholinergic-adrenergic transmission and adrenergic-cholinergic transmission.

Effects of depletion of catecholamines on the responses of effector organs to sympathomimetic amines.—Depletion of the transmitter from adrenergic nerve endings occurs following degeneration of the nerve or can be induced

by drugs such as reserpine and guanethidine. Following either type of procedure, there is enhanced responsiveness of the effector organs to exogenous epinephrine or norepinephrine (109, 125 to 135). However, there does not appear to be any direct quantitative relationship between drug-induced depletion of transmitter and supersensitivity of the end organ to norepinephrine. According to Kirpekar *et al.* (136), both surgical denervation and reserpine depleted almost completely the stores of catecholamines in the nictitating membrane. However surgical denervation caused a 200 to 300-fold increase in the sensitivity of the membrane to norepinephrine while the increase in sensitivity produced by reserpine was only 2-fold. Furthermore, degeneration of the preganglionic innervation of the membrane, which had no effect on the content of catecholamines in the membrane, increased the sensitivity of the membrane by a factor of 10 to 15-fold. Impairment of transmission at adrenergic junctions by reserpine and guanethidine must persist for several days before supersensitivity to norepinephrine can be observed (128, 129, 137). Muscholl (138) has suggested that reserpine enhances the responses of smooth muscle to norepinephrine by delaying its inactivation [Drug-induced denervation of peripheral organs has been reviewed by Emmelin (127).]

It has been known for sometime that following degeneration of the sympathetic nerve supply, the response of an organ to tyramine is diminished greatly or abolished completely. More recently, it has been demonstrated that treatment of animals with reserpine produces a similar decrease in the response of a variety of organs to tyramine (126, 130, 137, 139 to 146). Moreover, exposure of the reserpinized organ to norepinephrine restores the action of tyramine (e.g., 141, 147, 148). Finally, it also has been demonstrated that tyramine causes the liberation of catecholamines from some tissues (144, 149 to 155) but not others (152, 156). In guinea pig heart, the store from which tyramine liberates norepinephrine was about 20 per cent or less of the total stores of norepinephrine (157). In view of these findings, it seems reasonable to attribute the effects of tyramine to the releaseof catecholamines from adrenergic nerve endings, as proposed by Burn & Rand. In this connection, tachyphylaxis to ephedrine and tyramine (158, 158a) has been attributed to depletion of that bound norepinephrine which is available for release.

However, tyramine also has other actions at sympathetic neuro-effector junctions (144, 159). For example, the increase in the rate of contraction of the heart produced by dimethylphenylpiperazinium was associated with a considerably greater release of norepinephrine from the heart than was the same increase in heart rate produced by tyramine. These findings suggested a sensitizing action by tyramine of the cardiac response to endogenous norepinephrine (144). Conversely, Nasymth (160) has suggested that norepinephrine or its metabolites may facilitate the interaction between tyramine and adrenoceptive sites. It is of interest that a regulatory role in adrenergic transmission for metanephrine and normetanephrine also has been suggested (161).

Like surgical denervation, treatment of adrenergic neuro-effector junctions with cocaine potentiates the response of the junction to exogenous norepinephrine and epinephrine (e.g., 162 to 166). Earlier explanations of this action of cocaine included (*a*) inhibition of enzymes which destroy the catecholamines, and (*b*) sensitization of the effector organ by the reduction of the basal release of transmitter. Currently, the potentiation of catecholamines by cocaine is explained on the basis of a blockade by cocaine of nonspecific binding sites for norepinephrine around the nerve endings which results in an effective increase in the concentration of the amines in the junctional region (163 to 172). Stated briefly, this interpretation is based on the following observations, (*a*) cocaine decreased the uptake of catecholamines by various tissues (148, 153, 173); (*b*) cocaine potentiated the responses to catecholamines of innervated but not denervated structures (162, 163); and (*c*) the uptake of catecholamines by denervated effector organs was less than that of innervated organs (172, 174). It is also of interest that the potentiation by cocaine of the response to norepinephrine was greater than the potentiation of epinephrine (175). It is curious, however, that cocaine, unlike phenoxybenzamine (176) did not alter the amount of catecholamines recovered following stimulation of sympathetic nerves (165, 177). If interference by cocaine of nonspecific receptors is the basis for the potentiation, it might be expected reasonably that reduced tissue binding of norepinephrine would be reflected by an increased recovery of the amine.

In addition to cocaine, a number of other drugs (e.g., imipramine, pipradol, pheniprazine) also enhanced the responses of various organs to cathecholamines (87, 164, 175, 178). However, it is not clear if mechanisms similar to those stated above for cocaine underlie the potentiation produced by these drugs. Some of them, for example, differ from cocaine in that they also enhanced the responses evoked by tyramine (164).

Release of catecholamines from isolated chromaffin granules.—The effect of drugs on the uptake or release of norepinephrine by granules isolated from adrenergic nerves and the adrenal medulla serves as a model system for the study of some of the concepts mentioned above. ACh, nicotine, histamine, and 5-hydroxytryptamine have no effect on the liberation of norepinephrine from isolated granules (179, 180). These findings indicate that the liberation of catecholamines from the spleen and other structures (*vide supra*) produced by ACh and nicotine requires intact cells. On the other hand, the action of tyramine on the spontaneous liberation of norepinephrine from the granules is in general accordance with the actions of tyramine on intact cells. Tyramine, β-phenylethylamine, amphetamine, metanephrine, and reserpine, in high concentrations, accelerated the spontaneous release of norepinephrine from the granules (179, 181 to 184). However, there are some discrepancies between these studies and the ability of tyramine to cause the liberation of catecholamines from intact systems. For example, Stjärne (152) found that tyramine caused the release of norepinephrine into the venous effluent from the spleen but not from the adrenal medulla. As indicated above, tyramine

accelerated the release of norepinephrine from granules isolated from either splenic nerves or adrenal medulla. In addition, there are several conflicting reports of the actions of cocaine on the liberation of catecholamines from tissues by tyramine (149, 153), and of the effect of tyramine on the spontaneous release of catecholamines from chromaffin granules (180, 185). These differences may be related to the doses of drugs used. Also it is of interest that the actions of reserpine on the granules is dose-dependent; low concentrations inhibited the spontaneous release of norepinephrine (160) while high concentrations accelerated the release (182).

There has been a large number of studies which indicate that, in the chromaffin granules, catecholamines are bound to nucleotides (e.g., 186). Reserpine caused a depletion of both catecholamines and adenine nucleotides from avian adrenal medulla (187). However, the release of catecholamines was greater than that of the nucleotides. Similar studies with tyramine indicated that this agent increased the spontaneous liberation of catecholamines from chromaffin granules but had no effect on the spontaneous liberation of adenosine triphosphate. Under these conditions the uptake by the granules of tyramine was in stoichiometric proportion to the loss of norepinephrine (167). This latter observation indicates that an exchange reaction occurs in the process of tyramine induced liberation of catecholamines.

Metabolic aspects of adrenergic transmission.—An earlier account of the synthesis and enzymic destruction of catecholamines has been presented by Axelrod (188). In studies of the termination of the action of adrenergic transmitters, most of the emphasis has been placed on the enzymes monoamine oxidase and catechol O-methyltransferase. The various substances which inhibit monoamine oxidase are well known and require no additional comment. On the other hand, inhibitors of O-methyltransferase have been studied in detail only during the past three or four years (189, 190). Of these, pyrogallol has been the most commonly used inhibitor of this enzyme. The inhibition of O-methyltransferase by pyrogallol is reversible, and although there are some indications of a noncompetitive component in the reaction, it is primarily of a competitive nature (191). Since the amount of O-methyltransferase in tissues decreased following degeneration of sympathetic innervation, at least part of the content of the enzyme in tissues was probably associated with neural structures. Furthermore, the decrease in the enzyme did not appear to be related to denervation supersensitivity to catecholamines (192). Other characteristics of the enzyme and inhibitors of O-methyltransferase have been discussed by Axelrod (188, 193).

Pyrogallol and other polyphenols prolong moderately the responses of animals to both exogenous and endogenous catecholamines (194, 195). However, the actions of epinephrine were prolonged to a greater extent than were those of norepinephrine. Since norepinephrine was bound more firmly to tissues than epinephrine, the differential preservation *in vivo* by pyrogallol of the two amines can be attributed to differences in the exposure of epinephrine and norepinephrine to enzymatic destruction (194). The findings of

Axelrod & Tomchick (196) that the rate of metabolism of norepinephrine was increased following the administration of tyramine or other sympathomimetic amines also was explained on the basis of an interference by the amines with the binding of norepinephrine to tissues and, consequently, an increase in the exposure of norepinephrine to enzymic destruction. With the availability of inhibitors of both monoamine oxidase and O-methyltransferase, a number of studies have been made to assess the roles of the two enzymes in the termination of the actions of catecholamines (195, 197 to 199). When one of the enzymes was inhibited, the other was present in amounts adequate to metabolize circulating catecholamines (197). When both enzymes were inhibited simultaneously, metabolism of the circulating amines was depressed markedly, but a new degradation product was formed (197, 198). The identification and importance of the new metabolite remains to be determined.

Since pyrogallol increased the concentration of norepinephrine, and lowered the concentration of normetanephrine in almost all tissues, it is apparent that O-methylation occurs locally. Inhibition of monoamine oxidase had no effect on the tissue concentration of norepinephrine but increased the concentration of normetanephrine. Consequently, it was suggested that the primary function of monoamine oxidase is the deamination of the methylated catabolite of norepinephrine (171). The rate of release of norepinephrine from tissues also was important in determining whether the destruction of the transmitter was accomplished by monoamine oxidase or O-methyltransferase. Comparisons of the metabolism of norepinephrine intravenously injected and that released endogenously by reserpine or tyramine have indicated that O-methylation is the major process involved in the inactivation of circulating and tyramine-released norepinephrine, while the norepinephrine released more slowly by reserpine is metabolized primarily by deamination (154, 200). In conflict with these observations are those of Chidsey *et al.* (155) who found that the liberation of norepinephrine by tyramine was not accompanied by an increase in either normetanephrine or 3-methoxy-4-hydroxymandelic acid.

The suggestion also has been made that neither monoamine oxidase nor O-methyltransferase plays a physiological role in the inactivation of transmitter liberated at adrenergic junctions, but that the uptake of the released transmitter by local tissues plays the major role in the inactivation process (174, 195, 201 to 203). The findings that catecholamines are taken up rapidly by many tissues is compatible with this suggestion (e.g., 174, 195, 204, 205). Further, the observation by Stinson (199) that catecholamines did not appear in the perfusate of rabbit ears during sympathetic stimulation, even in the presence of inhibitors of monoamine oxidase and O-methyltransferase, can be explained on this basis.

Lockett had demonstrated previously that an isoproterenol-like compound was present normally in the adrenal medulla (206), was released in

the lungs during sympathetic stimulation (207), and appeared in arterial blood following a systemic injection of epinephrine (208). Consequently, Lockett has suggested that the isoproterenol-like substance was a metabolite of epinephrine. Since pretreatment of animals with pyrogallol prevented the occurrence of the isoproterenol-like material (209), and inhibitors of monoamine oxidase increased the amount of the substance appearing in arterial blood, it was suggested that O-methyltransferase played an important role in the synthesis of the material, and that monoamine oxidase was important in its destruction. The main site of synthesis of the isoproterenol-like substance was the liver (209).

Recent studies (210, 211) have indicated that the antihypertensive actions of *alpha*-methyl-dopa were not due to the inhibition of the decarboxylation of 3,4-dihydroxyphenylalanine to form ultimately norepinephrine, or of the decarboxylation of 5-hydroxytryptophane to form 5-hydroxytryptamine, as previously thought. Although this agent did inhibit the decarboxylation of aromatic amino acids (e.g., 210 to 214) and decreased the content of norepinephrine in the brain and heart, the tissue levels of norepinephrine remained low long after the normal activity of the decarboxylating enzyme had returned (210, 211). Furthermore, the biosynthesis of the catecholamine occurred during this time, but the ability of the tissues to retain the newly formed amine was imparied. Recent work has indicated that *alpha*-methyl-dopa was itself decarboxylated by the enzyme (215, 216). Similar studies have been made with α-methyl-*m*-tyrosine (217).

It has been well established that activation of the sympathetic nerves to the heart or the application of exogenous catecholamines increase the phosphorylase *a* activity of the heart (218 to 223). Stimulation of the heart by the cardiac glycoside, ouabain, or by 5-hydroxytryptamine did not increase phosphorylase *a* activity (222). On the other hand, stimulation of the vagus nerve or the injection of ACh resulted in a decrease in enzyme activity and antagonized the actions of McN-A-343 both on the enzyme activity and on the force of contraction of the heart (222). Therefore, Hess *et al.* (224) have suggested that the autonomic nervous system plays an important role in the regulation of cardiac phosphorylase activity. Ellis & Vincent (225) have made an essentially similar suggestion concerning the control of cardiac function by ACh. They found that ACh and dichloro*iso*proterenol antagonized the glycogenolytic, ionotropic, and chronotropic actions of epinephrine on the isolated heart and suggested that these observations represent a possible biochemical basis for the cardiac actions of ACh. Mayer *et al.* (226) have questioned the necessity for implicating a causal relationship between the actions of catecholamines on enzyme activity and the force of contraction of the heart. In the latter study, a separation of the two actions of epinephrine was observed under some conditions. In contrast, Hess *et al.* (227), using a wide range of doses, observed no dissociation between the increased force of contraction produced by epinephrine and the stimulation

of phosphorylase activity, and concluded that the two actions of epinephrine were related. Earlier reviews of the effects of catecholamines on carbohydrate metabolism have been presented by Ellis (228) and Sutherland & Rall (229).

Adrenoceptive sites and adrenergic blocking agents.—The classification of the diverse actions of sympathomimetic amines is based, in part, on the selective blockade of these actions by various adrenergic blocking agents. The effects of traditional blocking agents (e.g., phenoxybenzamine) are attributed usually to the blockade of *alpha* receptors; whereas, those produced by dichloro*iso*proterenol (DCI) are attributed to blockade of *beta* receptors [Ahlquist's (230) nomenclature]. The blockade of some responses to sympathomimetic agents (i.e., inhibition of intestinal motility) requires both types of blocking drugs and is said to be regulated by both *alpha* and *beta* receptors. Levy & Ahlquist (231, 232) have proposed that the reversal of the depressor response to ethylnorepinephrine be added as a criterion of the blockade of *beta* receptors. Although this classification of receptors and blocking agents is quite useful, the complexities it leaves unresolved are illustrated by the following findings. Bronchodilation produced by stimulation of the sympathetic nerves to the lungs or by epinephrine and norepinephrine was converted to bronchoconstriction by dichloroisoproterenol. The bronchodilation produced by isoproterenol was reduced, but not reversed, by dichloroisoproterenol (233). According to Ahlquist's classification, these findings indicate the existence of both *alpha* and *beta* receptors on bronchiolar smooth muscle and that isoproterenol can activate only the *beta* receptor. On the other hand, contraction of the iris dilator muscle by epinephrine, norepinephrine, or isoproterenol was not blocked by dichloro-*iso*proterenol but was sensitive to blockade by phenoxybenzamine (234). Thus, in the iris, *iso*proterenol causes activation of *alpha* receptors. Conversely, the increase in the force of contraction of the heart produced by epinephrine, norephinephrine, and isoproterenol was blocked by dichloro-*iso*proterenol but not by blocking agents such as phenoxybenzamine (235, 236). Still further, some blocking agents (methoxyphenamine, dihydroergotamine, and dihydroergocornine) exhibit blockade of both types of receptors (232). Accordingly, the possibility of classifying receptors exclusively on the basis of effects of blocking drugs is disputable.

Similarly, the inhibitory actions of phenoxybenzamine, and ergotamine (237 to 239) on a variety of metabolic responses evoked by epinephrine in intact animals and isolated preparations are difficult to fit into this pattern of receptor nomenclature.

The characteristic depressor response to isoproterenol was reversed by the prior administration of phenylephrine, ergotamine, or ephedrine (232, 240 to 242). The reversal of isoproterenol by these agents was antagonized by reserpine and choline 2,6-xylyl ether (240). Maengwyn-Davies *et al.* (240) attributed the reversal to a change in the peripheral adrenoceptive sites produced by the sympathomimetic amines. On the other hand, Levey & Ahlquist (232) concluded that the reversal was due to cardiac stimulation

by isoproterenol in the presence of peripheral vasoconstriction by phenylephrine and ergotamine.

Nerve Growth Factor and Development of the Sympathetic Nervous System

Levi-Montalcini & Angeletti (243) have reviewed the details of a decade of study which should provide an extraordinary approach to a better understanding of the physiology and pharmacology of the sympathetic nervous system. By the use of a protein growth factor derived from mouse salivary glands, mouse sarcomas, snake venoms, or sympathetic ganglia, it was possible to cause a rather selective hypertrophy of sympathetic ganglia either *in situ* or in tissue culture. Conversely, treatment of animals with the antiserum to the growth factor prevented the development of sympathetic ganglia. The amount of norepinephrine in ganglia of animals treated with the growth factor was increased 3- to 4-fold. It is of some interest that the growth factor did not influence the amount of norepinephrine in the adrenal glands (244). In immuno-sympathectomized animals, the amount of norepinephrine in the heart and other tissues was decreased (245). To date, the effects of drugs on the animals treated either with the growth factor or with the antiserum have not been described.

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